DETECTION OF STRESS IN POT PLANTS
J. Harbinson
Agrotechnological Research Institute
postbus 17
6700 AA Wageningen
Netherlands

Abstract
Stress is considered to be major problem for plants, whether they are in cultivation or growing in natural settings. Consequently there is a need to have some means of identifying stress. Recently developed techniques for measuring photosynthetic processes using non-destructive biophysical techniques, especially chlorophyll fluorescence, offer a powerful and convenient tool for probing the physiological state of the plant. They have also been exploited for the detection of stress, screening for stress tolerance, and for quality determination. The physiological basis, techniques and application of these techniques will be discussed. The problems associated with definition of stress will also be considered.

1. Introduction
  1.1 What is stress?
One of the greatest problems associated with any discussion of the occurrence and detection of stress in plants is finding a reliable definition of 'stress'. The lack of rigour surrounding this definition can be gauged from the common use of 'stress' to describe causes (eg 'a stressful environment') and effects (eg 'detection of stress in pot-plants') (in this article I will continue with this ambiguous usage). The best approach would be based on a classification of the physiological and developmental state of the plant. Environmental changes will produce changes in the state of the plant and if stress could be defined as a state or class of states, then its detection would be straightforward. The difficulty with this idealized approach is first determining the physiological state of the plant, and, second, deciding which states should be classified as stress. An alternate approach is one of pragmatism; the occurrence of stress is assumed whenever there is diminished yield, viability, quality, competitiveness etc. This is a convenient way of defining and identifying stress, but such definitions are not, however, universally applicable but relate to specific situations, so they are not reliable. For example, many plants that occur naturally in 'stressful' environments would grow better, and thus be more productive, in a more benign environment but they are most competitive in the stressful environment. So, from one point of view such plants are stressed because their productivity is reduced in their natural environment, but, on the other hand, this is the habitat to which they are best adapted in competitive terms as they can grow and reproduce effectively. The weakness of the pragmatic approach to stress is that the definitions are relative and stress is being defined operationally and not in terms of the occurrence of a specific physiological state (or states).

Though there are problems with defining stress it is clear that stress is a real problem. For example, it is evident that following exposure to extreme environmental conditions plants become injured and describing this is as stress is non-controversial. The problem of definition lies less with these extreme responses and more with deciding when a moderate deficiency or excess of some factor becomes a stress. For

ACTA HORTICULTURAE 405, 1995
POSTHARVEST PHYSIOLOGY OF ORNAMENTAL PLANTS
example, the growth of plants on many natural soils appears to limited by nutrient supply, but could they be usefully be described as stressed and, if not, how nutrient deficient would they have to become before they could be so defined? These problems have their origin in the poverty of our understanding of stress in terms of altered physiological states. This is not simply a philosophical problem; the better we understand stress then the easier it will be to identify, classify and prevent. Some plant signal/response systems are reasonably well understood, for example, that of ethylene (Picton et al., 1995), but there is currently no description of a physical stress that comprehensively relates its detection and signal transduction at the cellular level to subsequent changes in gene expression, development, metabolism and physiology. This is, however, an area of active research and it is clear that patterns of stress responses exist, for example chilling, hypersensitive responses to pathogens and pollution responses may, at least in part, share a common signal transduction pathway, that of superoxide/peroxide formation (Doke et al., 1994; Prasad et al., 1994). It is, however, not yet possible to classify all stresses in terms of detection mechanisms and physiological, biochemical and developmental responses, so it is not possible to identify the occurrence of stress in terms of particular syndromes of signals and responses associated with different environmental excesses or deficiencies.

1.2 The detection of stress

There still remains the practical need to identify stress in plants, a question that can be (easily) extended to include the often related factor of quality. In the absence of a better understanding of the processes by which plants sense and respond to environmental challenges, it is necessary to adopt a more empirical approach based on established correlations between physiology and environmental extremes. Many techniques have been exploited to achieve this end. The effects of stresses severe enough to cause tissue necrosis, such as chilling, freezing and heat stress, have frequently been monitored using, for example, electrolyte leakage or tetrazolium red reduction which measure cellular integrity and respiratory activity respectively. Chlorophyll fluorescence has a long been exploited to monitor this kind of extreme stress. Recently, however, chlorophyll fluorescence and related techniques have been developed into more sophisticated quantitative tools with which to monitor in vivo the activity of photosynthetic light-harvesting and electron transport (Genty et al., 1989; Harbinson and Hedley, 1993). These techniques have been progressively exploited as techniques with which to assess stress (and quality) in plants. This application depends on the role that photosynthesis plays in the operation of the whole plant in terms of the energy and carbohydrate needed for metabolism and growth, and the ease of use of the measuring techniques themselves.

2. The use of biophysical techniques in the measurement of photosynthesis

Various biophysical measurements of photosynthesis have been considered for use in stress detection, generally in relation to screening procedures. These include chlorophyll fluorescence (Smillie, 1979; Smillie and Hetherington, 1983), photoacoustic effects due to photosynthetic energy storage and oxygen evolution (Malkin and Canaan, 1994), and light-induced absorbance changes due to electron transport and charge movement in and around the thylakoid membrane (Smillie, 1979). Most of these techniques are unsuitable for routine use for technical reasons; they require apparatus that is fragile, difficult in application, or which requires special environmental conditions for its application. Only one technique, chlorophyll
fluorescence, has been widely applied, although another more recently developed technique which uses light induced absorbance changes around 820nm to measure the kinetics of electron transport (Harbinson and Hedley, 1989, 1993) is technically easy to apply and gives results that are potentially more useful than those obtained using chlorophyll fluorescence.

2.1 Chlorophyll fluorescence: the physiological basics

The theory and use of chlorophyll fluorescence has been thoroughly described (Briantais et al., 1986; Krause and Weis, 1991) so only a brief summary will be included here. Chlorophyll fluorescence arises largely from photosystem II (PSII), and, with the exception of changes due to protein phosphorylation, all the short-term changes in the variable component of chlorophyll fluorescence yield (these yields are measured on a relative basis) are due to changes in photosystem II. As a consequence of this association with PSII, fluorescence is used to probe the functioning of PSII and, importantly, it can be used to determine the quantum efficiency of PSII ($\Phi_{\text{PSII}}$) (Genty et al., 1989) under a wide range of conditions. Other parameters can be calculated from fluorescence data, such as the photochemical ($q_p$) and non-photochemical quenching ($q_{np}$) of chlorophyll fluorescence (and by implication the excitation in the PSII pigment bed) (Quick and Horton, 1984; Schreiber et al., 1986; Krause and Weis, 1991), which are useful for understanding the operation and regulation of PSII and which have been employed in stress, or stress-induced injury, screening and detection procedures (e.g. Havaux, 1989; Schapendonk et al., 1992; Oquist et al., 1993). Additionally, some parameters, such as Fv,Fm, though encountered in the literature (e.g. MacRae et al., 1986; Toivonen, 1992), are obsolete in terms of contemporary physiological research. Their use, in most cases, reflects a combination of the technical limitations of early measurement systems and a poverty of understanding of how to understand the changes in fluorescence yield that were observed.

It is now clear that changes in the variable component of fluorescence are largely due to three main factors (Quick and Horton, 1984; Briantais et al., 1986; Krause and Weis, 1991). First, is the degree of reduction of the primary stable electron acceptor of PSII, a specialized plastoquinol named QA. Its reduction closes the reaction centre making it incapable of further photochemistry until it becomes re-oxidized by electron transfer to the plastoquinol pool, and closure increases the yield of PSII fluorescence. So, when all the QA is oxidized the yield is at the minimum level (F0), and when it is all reduced the yield is at the maximum level (Fm). Second, it is due to the degree of non-photochemical quenching (NPQ); this is a rapidly reversible protective mechanism that can compete with photochemistry as de-excitation (or quenching) mechanism for excitation energy in the PSII pigment bed. As NPQ increases it decreases the fluorescence yield corresponding to any degree of reduction of QA, so the yield of fluorescence corresponding to the Fm yield is variable and depends on the strength of NPQ. The third factor is the degree of photoinhibition; this is a slowly reversible, or irreversible, loss of photochemical efficiency of PSII which is paralleled by a decrease of variable fluorescence in a similar to NPQ. The photochemical efficiency of PSII and the yield of chlorophyll fluorescence will be determined by the combined action of these processes (Quick and Horton, 1984; Briantais et al., 1986; Genty et al., 1989; Harbinson et al., 1989; Krause and Weis, 1991; Foyer and Harbinson, 1994).

The increased understanding of the processes that determine fluorescence yield,
coupled with technological and methodological improvements, has allowed the refinement of the selection and use of fluorescence parameters. Currently, only $\Phi_{\text{PSII}}$, $q_p$, variants of NPO, and Fv/Fm are commonly encountered in the literature, and of these probably $\Phi_{\text{PSII}}$ is the most widely used in studies using applied chlorophyll fluorescence. To use measurements of chlorophyll fluorescence to measure $\Phi_{\text{PSII}}$ it is necessary to make two measurements, one of the steady-state yield and another of the Fm yield when all the QA has been reduced by an intense pulse of light (fig. 1). It is the relative values of these yield that are used to estimate $\Phi_{\text{PSII}}$. Fv/Fm gives the maximum efficiency of PSII photochemistry (Lavorel and Etienne, 1977) and is linearly related to the quantum efficiency of O2 evolution (Björkman and Demmig, 1987) and thus CO2 fixation under strictly light-limited conditions. It is a simply measured parameter and is widely used in plant stress studies because following photoinhibition, frequently associated with, or even after high or low temperature stresses in darkness its value decreases (Oberhuber and Bauer, 1991; Ögren and Rosenqvist, 1992; Havaux, 1993, 1994; Krause, 1994; Tijskens et al., 1994). It is obtained as the ratio between Fv (difference between F0 and Fm) and Fm on dark-adapted material (fig. 1).

2.2 Chlorophyll fluorescence measurement systems

Chlorophyll fluorescence measurement systems fall into three main classes: those in which a single light source serves as the actinic and measuring irradiance, and those in which separate sources are used and the measuring irradiance is modulated, and lastly fluorescence imaging systems. The first class of measurement is really only useful for measuring Fv/Fm, the some obsolete parameters, such as Fv/medium or some other parameter that can be measured during the photosynthetic induction process in dark adapted leaves.

The second class of measuring system is more complex in design and use but is more powerful. It allows $\Phi_{\text{PSII}}$ to be measured under conditions of continuous actinic irradiance, which can include direct full sunlight, and under more controlled conditions allows changes in $\Phi_{\text{PSII}}$ to be analyzed in terms of its components (Genty et al., 1989; Harbinson et al., 1989). A measurement of $\Phi_{\text{PSII}}$ under continuous irradiance allows the steady-state operation of photosynthesis to be monitored (Genty et al., 1990; Harbinson et al., 1990) and makes possible more subtle analyses of plant stress (van Kooten and Peppelenbos, 1993).

Fluorescence imaging in conjunction with further processing to produce images of $\Phi_{\text{PSII}}$ was first demonstrated in 1989 (Daley et al., 1989). More recently, improved techniques for obtaining such images have been developed that allow $\Phi_{\text{PSII}}$ to be mapped in a simpler, more reliable way (Genty and Meyer, 1994; Siebke and Weis, 1995) and it likely that imaging techniques will open many new possibilities for the assessment of stress and the consequences for photosynthesis.

2.3 Light-induced absorbance changes around 820nm, a window on PSI

Measurements of light-induced absorbance changes around 820nm ($\Delta A_{820}$) are used to estimate the relative oxidation state of $P_{700}$, the specialized reaction centre chlorophyll of photosystem I. The theory and general use of this technique has been described (Weis et al., 1987; Harbinson and Hedley, 1993; Klughammer and Schreiber, 1993; Harbinson, 1994) and it allows the calculation of the quantum yield of photosystem I ($\Phi_{\text{PSI}}$) and the rate constant for electron transport (Harbinson and Hedley, 1989). As $\Phi_{\text{PSI}}$ is nearly always linearly related to $\Phi_{\text{PSII}}$ (Weis et al., 1987; Harbinson et al., 1989, 1990; Genty et al., 1990; Peterson, 1991; Klughammer and
Schreiber, 1993) and is a more difficult measurement to make, there is little point in using this technique in place of $\Phi_{PSII}$ if all that is required is a measure of the photochemical efficiency of photosynthetic energy transduction. In contrast, the measurement of the rate constant for photosynthetic electron transport cannot be done using measurements of chlorophyll fluorescence but is a relatively easy measurement to make using $\Delta A_{820}$. The principle is simple; $P_{700}^+$ is formed in the light, and following the removal of the light this pool of $P_{700}^+$ is reduced, a process that can be recorded using the $\Delta A_{620}$ (Harbinson and Hedley, 1989; Harbinson, 1994) (fig 2). The reduction proceeds with a half-time of >4 ms and is mono-exponential, making it easy to obtain a rate constant for the process (Harbinson and Hedley, 1989; Schreiber et al., 1989; Harbinson, 1994). An alternative approach is produce an excess of $P_{700}^+$ using a short flash (approx. $10^{-3}$ s long), and then to measure the rate of reduction of the excess $P_{700}^+$ as the electron transport chain returns to steady-state (fig 2). This technique is more suited to measurements of $P_{700}^+$ turnover under conditions where there is no control of the ambient lighting but is still in need of further development. The rate constant for $P_{700}^+$ reduction is significant because it represents the rate of electron transport from the plastoquinol pool to $P_{700}^+$ and thus includes the rate limiting step for electron transport, the transfer of reducing equivalents from plastoquinol to the cytochrome $b/f$ complex (Genty and Harbinson, 1995). This is the step at which photosynthetic electron transport is regulated to co-ordinate it with metabolism and its rate constant therefore mirrors the demands of photosynthetic metabolism, and of CO$_2$ fixation when the stomata are open (Foyer et al., 1990; Genty and Harbinson, 1995). It is therefore a measure of the control that is applied to the photosynthetic energy transducing system, whether by short term physiological mechanism or long term developmental mechanisms.

When measured following a light-dark transition the rate constant for electron transport is independent, or nearly independent, of the irradiance (Harbinson and Hedley, 1989; Harbinson, 1994; Laisk and Oja, 1994) (fig 3). At low irradiances, where the small size of the $\Delta A_{820}$ implies only a slight accumulation of $P_{700}^+$, the rate constant for the relaxation of the $\Delta A_{820}$ following a light-dark transition may be smaller than that obtained at higher irradiances. However, in leaves exposed to a low steady-state irradiance the rate constant obtained from a $\Delta A_{820}$ relaxation following flash induced oxidation of $P_{700}$ (which represents the turnover a larger fraction of the total $P_{700}$ pool) is higher than that obtained during a dark-light transition. The small rate constant obtained at low light levels may, therefore, be due to a small component of the total $P_{700}$ pool that is atypical in some way; in leaves exposed to a low steady-state irradiance the rate constant obtained from a $\Delta A_{820}$ relaxation following flash induced oxidation of $P_{700}$ (which represents the turnover a larger fraction of the total $P_{700}$ pool) is higher than that obtained during a dark-light transition.

Given its relationship to the control and co-ordination of photosynthetic electron transport, a correlation might be expected between its rate constant and the maximum rate of CO$_2$ fixation for a leaf, and they are in fact simply related (Laisk and Oja, 1994; Genty and Harbinson, 1995). What is surprising is that the data for most leaves so examined, including those with dissimilar photosynthetic rates and morphologies, have the same relationship (unpublished observations). Such a consistent correlation is surprising as the rate photosynthetic electron transport is, in principle, determined by other factors in addition to the rate constant for electron transport, such as the total amount of $P_{700}$. The basis of the correlation therefore
deserves further investigation. In conclusion, the rate constant for $P_{700}^+$ reduction has potential as a simple means with which to measure the photosynthetic capacity of leaves, certainly on a relative basis and possibly on an absolute basis. Its independence of irradiance implies that no control need be exerted over this factor during the measurement (unlike measurements of photochemical efficiency, which are irradiance dependent). The measurement can also be applied to dark-adapted photosynthetic material, where it may have value in determining the developmental state of the material.

3. Application of the techniques

Examples of the use of non-destructive measurements of photosynthesis to identify stress, stress tolerance and quality can be divided into two main classes; those that use the Fv/Fm or some other parameter measured on dark adapted leaves and those that use a measurement of $\Phi_{PSII}$ or some other parameter within an irradiance treatment. Because of recent consolidation of the range of parameters derived from chlorophyll fluorescence measurements, parameters now considered to be obsolete will often be encountered in older papers. These are frequently difficult to rigorously interpret in terms of photosynthetic physiological processes even in the light of current understanding of photosynthetic operation and regulation. Nonetheless, it is clear that they often reported significant differences between species or treatments which would also be expected to be apparent if more modern techniques had been used.

Most applications of chlorophyll fluorescence to plant stress measurement or screening for tolerance involve crop plants or model species such as spinach. Of these the emphasis has usually been with developing screening techniques for stress tolerance, and though this is not the same as measuring or detecting stress, it is close enough to be relevant. In many cases fluorescence has been used to monitor damage to the photosynthetic energy transduction system itself (a role for which it is very well suited) and not to measure plant stress in general. To achieve the latter, it is necessary to measure both some fluorescence parameter and correlate its value with an independent estimate of injury, performance or quality. It is surprising how infrequently this has been done and this is still an area in need of more experimentation. Nonetheless, the examples in the next section should serve to illustrate what is achievable. There are no examples using either measurements of $P_{700}^+$ reduction rate of fluorescence imaging to screen for stress or detect it in plants under field conditions; both of these techniques are so novel that neither has been extensively exploited.

3.1 Measurements on dark-adapted material

To the present, most published uses of chlorophyll fluorescence in stress detection have used measurements on dark-adapted material. Included in these measurements are the detection of damage provoked by chilling, freezing, ice, heat and high light in crop plants, including horticultural species (eg Smillie, 1979; Smillie and Hetherington, 1983; MacRae et al., 1986; Yakir et al., 1986; Bodner and Larcher, 1989; Havaux, 1989; Walker et al., 1990; Groom and Baker, 1992; McMahon et al., 1994), and chilling and freezing stress in model species (eg Somersalo and Krause, 1989). Some measurements of photoinhibition have also been made on plants growing in natural situations (eg Groom et al., 1991; Ögren and Rosengqvist, 1992; Ball, 1994). Only occasionally has a fluorescence response been correlated to another index of
stress tolerance either qualitatively (Smillie and Hetherington, 1983; MacRae et al., 1986; Bodner and Larcher, 1989; Havaux, 1989; Walker et al., 1990) or quantitatively (Kamps et al., 1987). In general, these data show that fluorescence measurements can distinguish between stress-sensitive and tolerant species, at least at a gross level, though MacRae et al. (1986) found that their assay did not always distinguish between chilling tolerant and sensitive species and McMahon et al. (1994) only found an effect of chilling on their fluorescence parameter in Episcia but not in Dieffenbachia. Similarly, in a study on the chilling sensitivity of different Saintpaulia spp, Bordner and Larcher found that though Fv/Fm could not be used to distinguish between species with different degrees of chilling tolerance, they were, with the exception S. shumensis, capable of being distinguished using measurements of fluorescence parameters at steady-state (Bodner and Larcher, 1989). Fluorescence measurements made on dark-adapted material has been used to measure chilling injury to green peppers (Lurie et al., 1994; Tijskens et al., 1994) and cucumber (Tijskens et al., 1994) during cold storage. Broccoli ripeness may also be determined using fluorescence measurements (Toivonen, 1992), as may leaf senescence in cut flowering shoots of Alstroemeria (Jordi et al., 1994).

In all the above examples the stresses imposed were relatively severe, and, in some cases (Bodner and Larcher, 1989; McMahon et al., 1994), produced necrosis of the material. This class of measurement is most effective at detecting when plants have been exposed to conditions close to their survival limits, but not for detecting the more subtle physiological changes associated with the concept of internal quality. Exceptions to this are the measurements on cold stored chilling sensitive vegetables and changes associated with the ripening and senescence of at least some vegetables or cut flowers.

3.2 Measurements of chlorophyll fluorescence made at steady-state

This is a potentially more sensitive technique, measuring as it does the energetic demands of photosynthesis and its regulation. Though measurements of chlorophyll fluorescence at steady-state have been used extensively to probe the operation of photosynthetic electron transport and its relationship to CO₂ fixation, photorespiration and photosynthetic metabolism, little has yet been published in which these measurements have been employed to assay the degree of stress or the quality of plants. However, based on what is known about photosynthetic electron transport is regulated it is possible to suggest ways in steady-state chlorophyll fluorescence measurements could be used to give physiological information about the plant and thus to detect stress etc.

Using a technique in which Φₚₛᵢᵳ was measured 4 mins after an increase of the irradiance of leaves that had been allowed to reach steady-state at an irradiance of 12 μmol m⁻² s⁻¹, van Kooten and Peppelenbos (1993) were able to estimate the quality of chrysanthemum cuttings. This technique measures the progress of photosynthetic induction, and though its biochemical or physiological basis has not yet been determined it has been applied more generally and successfully as a probe for quality measurement in pot plants.

Treatments which are known to produce decreases in the rate of CO₂ fixation (Yakir et al., 1986; Sassenrath and Ort, 1990) will also produce effects on Φₚₛᵢᵳ (Genty and Harbinson, 1995), and thus insofar as the decreases in the rate of CO₂ fixation is in response to stress, then Φₚₛᵢᵳ could be used to detect that stress (eg
Harbinson and van Vliet, 1992). A further possibility is the detection of the sink-limitation of photosynthesis which can develop in response to carbohydrate accumulation by the plant. Source-sink balance may be implicated in floral initiation and development and so may be useful quality parameter. Various techniques exist for the detection of the sink-limitation of photosynthesis, and a simple technique is to measure the photosynthetic response following the elimination of photorespiration. In the absence of any limitation, this should produce an increase of CO₂ fixation at any irradiance level by ~1.4 and the Φₚₛᵢᵢ/irradiance relationship should remain unaltered, whereas in the presence of sink-limitation the increase in the rate of CO₂ will be smaller or even non-existent and Φₚₛᵢᵢ will decrease more with increasing irradiance (Genty et al., 1990; Harbinson, 1994). An example of this type of response can be seen in fig. 3, which shows the relationship between Φₚₛᵢᵢ and irradiance for Begonia luzonensis leaves grown under high and low irradiance, with the leaf from the high light environment showing much more marked sink-limitation of photosynthesis than that from the low light environment. The usefulness of an approach of this kind has yet to be demonstrated on a real product, but as our understanding of the regulatory relationships within photosynthesis increase it is likely that other such techniques will be developed.

3.3 Application of measurements of electron transport kinetics

There are no examples where measurements of electron transport kinetics via ΔAₖ₂₀ measurements have been formally used to identify stress, tolerance or quality. Nonetheless the potential of this technique, as determined from physiological experiments is clear. An example of the application of P₇₂₀⁺ kinetics to measure the internal state of the plant can be seen in the responses of leaves with sink-limited photosynthesis. In the sink-limitation example used in the previous section (fig 3), the changes in the irradiance response of Φₚₛᵢᵢ were paralleled by changes in the rate constant for electron transport (fig. 3). As a general conclusion, it seems that the P₇₂₀⁺ reduction rate can be used in the same way as Φₚₛᵢᵢ measurements.

4. Conclusion

It seems apparent that stress is difficult to define rigorously, and this requires that careful thought be given to the purpose of identifying stress, the means by which it is identified, and the broader meaning that may be attached to the results. Non-destructive biophysical measurements of photosynthesis offer an attractive means of detecting stress in plants. The equipment is easy to use, the results now have quantitative physiological meaning, and there usefulness in this application has been demonstrated. Developments are imminent not only in our understanding of how plants sense and respond to their environment and develop the symptoms of stress, but also in the range and usefulness of instruments that can be used to detect stress and screen for stress tolerance and quality.

Acknowledgements
I would like to thank Drs W van Doorn and C. van der Schoot for helpful discussions that contributed substantially to this article.

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Fig 1. An overview of chlorophyll fluorescence changes in vivo measured using a modulated system (such as a PAM) during photosynthetic induction and steady state. With the activation of the measuring beam, the relative fluorescence yield rises to the F₀ level. If the leaf has been sufficiently dark-adapted to allow the relaxation of all non-photochemical quenching (typically requires 20 mins) applying a saturating pulse will maximally increase the yield to the Fₘ level. Fᵥ/Fₘ can be calculated from both F₀ and Fₘ as (Fₘ - F₀)/Fₘ. The start of actinic light will produce an increase in the fluorescence yield to P, the value of which depends on the intensity of the actinic light; if this is intense enough, P may be as high as Fₘ. With time after the start of the actinic irradiance steady-state photosynthesis is achieved. This may take many tens of minutes depending on the leaf and its history. The fluorescence increase in response to saturating pulse under these conditions can be used to estimate Φₚₛᵢ. The application of the saturating pulse (an irradiance of 7500 - 10 000 μmol m⁻² s⁻¹ is normally required) will again produce an increase in the fluorescence yield. In this case the value of Fₘ is lower than during the dark-adapted condition because of the action of non-photochemical quenching. Φₚₛᵢ is calculated from ΔF/Fₘ where ΔF is the fluorescence increase produced upon adding the saturating pulse.
Fig 2. The changes of the $\Delta A_{820}$ and thus the pool of $P_{700}^+$, in a *Stephanotis floribunda* leaf to (left) a 1 ms flash with an irradiance of 3 000 $\mu$mol m$^{-2}$ s$^{-1}$ superimposed on a background irradiance of 240 $\mu$mol m$^{-2}$ s$^{-1}$, and (right) the cessation of the background irradiance of 240 $\mu$mol m$^{-2}$ s$^{-1}$. In both cases $P_{700}^+$ oxidation precedes mono-exponentially and the fitted first-order rate constants for the absorbance changes are shown. In each change the direction of the absorbance change corresponding to $P_{700}^+$ reduction is shown.
Fig 3. A comparison of the $\Phi_{\text{PSII}}$ /irradiance (A, B) and rate constant/irradiance (C, D) (the rate constant for the $\Delta A_{520}$ relaxation and thus $P_{700}^{+}$ reduction) relationships for leaves of *Begonia luzonensis* grown in high light (150 $\mu$mol m$^{-2}$ s$^{-1}$) and low light (10 $\mu$mol m$^{-2}$ s$^{-1}$), and measured in gaseous phases comprised of 350 ppm CO$_2$, 2% O$_2$ remainder N$_2$ (●) or 350 ppm CO$_2$, 20% O$_2$ remainder N$_2$ (○). This choice of gaseous phases was chosen to reveal the presence of sink-limitation of photosynthesis, which is revealed by the greater loss of $\Phi_{\text{PSII}}$ with increasing irradiance in the 2% O$_2$ environment compared to the loss in the 20% O$_2$ environment. In this case the plants grown in low light were non-flowering and not so sink-limited as the flowering plants grown in the high light environment.